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In Vitro Transcription (IVT) Cas9 or Cas9-mSA mRNA for microinjection

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An abbreviated version of this protocol was published in eLIFE in Jul 2017

AIRE is a critical spindle-associated protein in embryonic stem cells

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Detailed protocol

Cas9 is cloned into the pCS2 IVT plasmid containing an SP6 promoter.

Linearize plasmid using NotI-HF.

Run 5µl on gel to check complete linearization. Purify rest by ethanol precipitation. Resuspend in RNase-free water. Quantify using Nanodrop.

Cas9 is then in vitro transcribed using the Ambion mMessage mMachine SP6 Kit according to the manual.

NCAP	20µl
10x buffer	4µl
2µg linearized Cas9-pCS2	x µl
ddH$_2$O	to 40µl
total	40µl

Incubate >4 hours 37°C.

Add 2µl Turbo DNase and incubate 15 min 37°C.

Cas9 mRNA purification:

For RNA cleanup use RNeasy Mini Kit (Qiagen, catalogue number 74106). Elute in 30-40µl RNase-free water.

Quantify using Nanodrop, yield should be between 600-1200ng/µl.

Run 2µl on gel to check integrity.

Aliquot Cas9 mRNA and store at -80°C.

How to cite:（Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used）

1. Gu, B. and Rossant, J. (2019). In Vitro Transcription (IVT) Cas9 or Cas9-mSA mRNA for microinjection. Bio-protocol Preprint. bio-protocol.org/prep103.
2. Gu, B., Lambert, J., Cockburn, K., Gingras, A. and Rossant, J.(2017). AIRE is a critical spindle-associated protein in embryonic stem cells. eLIFE. DOI: 10.7554/eLife.28131

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